Ecology, 93(1), 2012, pp. 46–55

A test of the senses: Fish select novel habitats by responding to multiple cues

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Abstract. Habitat-specific cues play an important role in orientation for animals that move through a mosaic of habitats. Environmental cues can be imprinted upon during early life stages to guide later return to adult habitats, yet many species must orient toward suitable habitats without previous experience of the habitat. It is hypothesized that multiple sensory cues may enable animals to differentiate between habitats in a sequential order relevant to the spatial scales over which the different types of information are conveyed, but previous research, especially for marine organisms, has mainly focused on the use of single cues in isolation. In this study, we investigated novel habitat selection through the use of three different sensory modalities (hearing, vision, and olfaction). Our model species, the French grunt, *Haemulon flavolineatum*, is a mangrove/seagrass-associated reef fish species that makes several habitat transitions during early life. Using several in situ and ex situ experiments, we tested the response of fish toward auditory, olfactory, and visual cues from four different habitats (seagrass beds, mangroves, rubble, and coral reef). We identified receptivity to multiple sensory cues during the same life phase, and found that different cues induced different reactions toward the same habitat. For example, early-juvenile fish only responded to sound from coral reefs and to chemical cues from mangroves/seagrass beds, while visual cues of conspecifics overruled olfactory cues from mangrove/seagrass water. Mapping these preferences to the ecology of ontogenetic movements, our results suggest sequential cue use would indeed aid successful orientation to novel key habitats in early life.

Key words: behavior; coral reef fish; French grunt; habitat selection; Haemulon flavolineatum; *olfaction; ontogenetic shifts; sound; vision.*

Introduction

The ability of animals to acquire information from their environment is essential for orientation toward suitable habitats, and ultimately influences fitness and survival. Multiple environmental cues, both biotic and abiotic, can guide animals through a mosaic of different habitats. To act upon these cues, sensory systems must develop and tune in on accurate and relevant information, which will vary among species, and may change for different life phases within a species. The use of multiple cues for orientation has been shown for various terrestrial animals (e.g., birds [Åkesson and Hedenström 2007]; insects [Renwick and Radke 1988, Brevault and Quilici 2010]; mammals [Rossier et al. 2000]). In the marine environment, animals encounter a different suite of cues to those on land. Differences in water chemistry, wave motion, currents, magnetic fields, light, and variation in ambient noise are examples of possible orientation cues in water (Lohmann et al. 2008*a*). These stimuli vary in concentrations, gradients, and intensities on temporal and spatial scales throughout the seascape. Many taxa are known to use sensory information for short- and long-distance orientation (moving relative to the environment) and navigation (movement toward a goal), including whales (Walker et al. 1992), turtles (Avens and Lohmann 2003), sharks (Collin and Whitehead 2004), salmonid fish (Dittman and Quinn 1996), reef fish (Myrberg and Fuiman 2002), and crabs (Radford et al. 2007).

The attractiveness of habitat-specific cues can be positively affected by early experience in dispersing animals (Aubret and Shine 2008). Moreover, the fitness of an animal can be greatly enhanced if natal experience provides an accurate estimate of habitat quality (Stamps and Davis 2006). The successful return to natal habitat by use of different environmental cues is observed in both terrestrial (e.g., lizards [Freake 2001]; pigeons [Walcott 2005]; ants [Steck et al. 2009]) and marine (e.g., salmon [Dittman and Quinn 1996]; reef fish [Dixson et al. 2008]; sea turtles [Lohmann et al. 2008*b*]) species.

Manuscript received 22 November 2010; revised 20 June 2011; accepted 15 July 2012. Corresponding Editor: J. R. Rooker.

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These homing mechanisms are often based on the idea that larvae or juveniles imprint on habitat cues associated with their natal origin, and utilize this prior experience to return to the same areas as their parents. However, our understanding of the abilities of animals to use environmental cues to find novel habitats without prior experience remains limited. This ability is critical for the many organisms that have a dispersive larval life stage, but do not have the opportunity to imprint on habitat cues prior to their dispersal away from adult reproductive habitats.

Theory suggests that for locating novel habitat, multiple cues are used simultaneously or in sequential order, depending on strengths of gradients and the distance from the source (Kingsford et al. 2002). While sound and chemical cues can produce gradients on large spatial scales (kilometers), visual cues are useful only at small distances (a few meters) (Kingsford et al. 2002, Leis and McCormick 2002). Besides cues emitted by habitats, conspecifics and heterospecifics also provide cues to which animals can respond (Gebauer et al. 2002, Lecchini et al. 2005; Huijbers et al. 2011). Since different cues operate over different spatial scales and habitat requirements change with ontogeny, sensory preferences of animals may adapt according to their life stage and local sensory environments.

The majority of demersal marine organisms, including almost all of the rich diversity of fauna found on coral reefs and associated tropical coastal ecosystems, display a two-phase life history strategy with a potentially dispersive oceanic larval stage followed by a sedentary adult phase (Caley et al. 1996). Thus, almost without exception, reef animals including fish face the challenge of finding suitable benthic habitat after completing an offshore larval phase. During their pelagic phase, coral reef fish larvae quickly transform from plankton into competent swimmers with well-developed receptor organs for sensing their environment, attributes which in combination could enable orientation toward specific settlement sites (Leis 2006). A suite of tropical reef fish species have been shown to respond toward acoustic, visual, or olfactory cues to locate reefs (Atema et al. 2002, Lecchini et al. 2005, Simpson et al. 2005, Montgomery et al. 2006), and on a smaller spatial scale to discriminate between habitats (Lecchini et al. 2007, Huijbers et al. 2008) and find conspecifics (Døving et al. 2006, Igulu et al. 2011). Despite the fact that multiple cues potentially influence behavior in an additive or consecutive manner, previous research on coral reef fish has mainly focused on the use of single cues in isolation. Hence our understanding of potential multiple cue use and the possible sequence or hierarchy of cues used remains rudimentary.

In the current study, multiple cue use by early juveniles was investigated using a mangrove/seagrass-associated reef fish species: the French grunt, *Haemulon flavolineatum*. This species displays sequential ontogenetic habitat shifts from the open ocean, where embryonic and larval development take place, first to coral rubble settlement habitats in back-reef areas, then to seagrass and mangrove juvenile habitats, and finally to coral reefs (Grol et al. 2011*b*), making it ideal for the investigation of multiple cue use for selecting novel habitat. Previous studies have shown that larval fish that settle directly on coral reefs can respond to auditory (Simpson et al. 2005), olfactory (Atema et al. 2002), and visual cues (Lecchini et al. 2007) emanating from reefs. However, such cues do not provide sufficient relevant information to larvae that settle and spend part of their life cycle in nonreef environments. We therefore tested the hypothesis that early juvenile French grunts possess adaptive orientation behavior, and tested for directional responses to multiple cues from mangroves and seagrass beds as well as from coral reef habitat. Multiple in situ and ex situ choice experiments were designed to test for responses to (a) habitat-specific sound, (b) habitat-specific visual structures, and (c) habitat-specific olfactory cues. Additionally, the interactive effect of visual cues from conspecifics with olfactory habitat cues was tested to explore hierarchy or conflict between multiple cues. The findings of this study provide much-needed information on the mechanisms and adaptive behavior that enable successful orientation toward suitable juvenile habitat, and highlight how young reef fish may utilize all the information available to find a sequence of habitats before recruiting to their final adult habitat.

Materials and Methods

Study site and species

The French grunt, *Haemulon flavolineatum,* is one of the most common fish species in the Caribbean, and shows ontogenetic movements among coastal habitats (Nagelkerken 2007). In this study, fish with a standard length of 8–30 mm were collected from a sand/rubble habitat in the mouth of Spanish Water Bay and Piscadera Bay on the Caribbean island of Curaçao, Netherlands Antilles (12°07′ N, 68°55′ W), where settlement of this species in Curaçao is at naturally high levels. At sizes below 5 cm total length, French grunts are diurnally active zooplanktivores, in contrast to larger fish that are typically nocturnal zoobenthivores (Verweij et al. 2006). Fish were caught with a fyke net at 3–4 m water depth and transported in buckets with fresh seawater from the collection site to the laboratory at the end of the day prior to each experiment. Fish were housed overnight in aerated flow-through aquaria and were not fed. Each fish was only used once in one cue experiment, and fish were tested individually in all experiments. Fish that were used for acoustic experiments were housed without bubblers, as the acoustic conditions of the housing environment can influence subsequent behavior in test arenas (Simpson et al. 2010), and may cause temporary damage to their hearing (Tolimieri et al. 2004).

Ex situ olfactory cue experiments were carried out in the outdoor laboratory of the Carmabi Institute, which

is located next to Piscadera Bay. A shallow, sandy flat in front of the laboratory was used for in situ cage experiments, in which the response to visual and acoustical cues was studied. All experiments were carried out during daylight hours between 0900 and 1800 hours.

Experimental design

Three different choice experiments were designed to investigate the response of fish toward acoustic, olfactory, and visual cues from specific habitats. Two of these cues were tested in an in situ setup in which other cues were excluded, and thus the effect of that particular cue alone was examined. The first in situ experiment tested if fish display a directional response toward habitat-specific sounds from four different habitats (seagrass beds, mangroves, rubble, coral reef). This study was conducted with one type of sound per trial, whereas the other two experiments with visual and olfactory cues tested competing stimuli. The second in situ experiment tested the response toward visual habitat cues using a multiple-choice design with four different microhabitats (rubble, coral reef, mangrove, and seagrass) presented simultaneously. Subsequently, the experiment was conducted with visual cues from microhabitat vs. conspecifics or heterospecifics. Olfactory cues were tested ex situ using a flow tank in which two different water types (bay water vs. coral reef water) were offered to the fish. In this last experiment, conspecifics were visible on the sides of the flow tank, testing the interactive effect of conflicting olfactory habitat cues and visual conspecific cues.

Behavioral arena: acoustic cues

We designed an acoustically transparent 2.5 m long cylinder (0.35 m diameter) from wire mesh (5-mm mesh size) (see Appendix A), which was placed at a depth of ~2 m above a sandy bottom >50 m away from any vegetated or reef habitat. Electrovoice UW-30 underwater speakers (frequency response 0.1–10 kHz; Lubell Labs, Columbus Ohio, USA) set to broadcast sound ~10–15 dB above ambient levels at 105 dB re 1 μPa at 1 m) were placed on either side of the cage. The sounds that were broadcast by the speakers were recorded in each of the four habitats (seagrass, mangrove, rubble, reef) using a calibrated omnidirectional hydrophone (HiTech HTI-96-MIN with built-in preamplifier; High Tech Incorporated, Gulfport, Mississippi, USA) and an Edirol R-1 24-bit recorder (44.1 kHz sampling rate; Roland Systems Group, Bellingham, Washington, USA; gain level calibrated using a 1 kHz sine wave produced by a signal generator and measured with an oscilloscope during the recording). To avoid potential pseudoreplication introduced by using a single recording in playback experiments (Slabbekoorn and Bouton 2008), the recording of each habitat consisted of six 3-minute sound fragments (see Appendix B). These fragments varied with time of day, season, and water depth, and ensured that we were investigating a general response by fish to the noise of each type of habitat. There was clear heterogeneity in the sounds recorded in the four different habitats (one-way ANOVA of rms broadband intensity, $F_{3,18} = 8.87$, $P = 0.001$), characterized by higher broadband intensity of sound in hard-substrate (reef and rubble) habitats, higher levels of low-frequency (100–1000 Hz) sounds in the hard-substrate environments (which are likely to be fish vocalizations [Simpson et al. 2008]), and peaks in higher frequency noise likely to be invertebrate noises in soft-substrate habitats at 1100–1200 Hz and in hard substrates at 1500–1700 Hz (see Appendix C). This suggests that the different habitats all had distinct characteristics and that the recordings were characteristically different as treatments for choice chamber experiments.

In each experiment only one fish was tested at a time in the chamber. In total 50–58 fish were tested per habitat-specific sound. During a trial, sound from only one type of habitat was played by one speaker, and the speaker on the opposite side of the cage was silent and acted as a control. Habitat sound treatment and broadcasting speaker side were changed randomly after each experiment to rule out any cage effects. We did not use a choice test between two different sounds to avoid creating a very artificial scenario with two acoustic gradients from contrasting but very nearby habitats.

To determine the gradient in sound intensity in the cage, we took recordings during playback at five positions along the experimental set-up, and used Avisoft-SASLab Pro (Avisoft Bioacoustics, Berlin, Germany) to calculate root mean square broadband noise levels at each location. If a cylindrical model of sound propagation (which is well suited to the shallow-water environment of the study [Au and Hastings 2009, Simpson et al. 2010]) is used to estimate the gradient of sound in the chamber, the prediction is that at between 5 and 7.5 m from the speaker (see Appendix A for chamber configuration), there would be a 1.75 dB decrease in the sound level within the chamber. Recordings taken on four occasions during playback of reef noise along the axis of the chamber matched this prediction with a mean drop in the sound level of 1.78 dB.

The cage was visually divided into three sections: high sound, middle, and low sound. At the start of an experiment when the sound started playing, one fish was introduced into a small wire cylinder at the center of the cage and allowed to acclimatize for a period of three minutes. The small cylinder was removed after the acclimatization period and the behavior of the fish was observed from a distance of 2–3 m perpendicular to the middle of the cage for 15 minutes using snorkeling gear, and the time spent by each fish in each section of the cage was recorded.

Behavioral arena: visual cues

A square cage ($1.0 \times 1.0 \times 0.7$ m; see Appendix A) was constructed with iron rods (8 mm diameter) and covered

with wire mesh (5-mm mesh size), except for the bottom part, which rested at a depth of ~4 m on the sandy substratum, to test the response of focal fish toward visual cues. Triangular boxes, made with Plexiglass, with a transparent glass front pane, were placed in each corner of the cage, surrounding a large central sandy area without any visual structures. The tops of the boxes were closed with lids during the experiments to ensure that chemical cues did not exude from the contents of the box. Between experiments the lids were opened to provide fresh seawater to each box.

In the first series of experiments each box contained one of four microhabitats. These microhabitats were constructed with live pieces collected in the field, namely mangrove roots (*Rhizophora mangle*), seagrass plants (*Thalassia testudinum*), rubble (small pieces of dead and broken coral), and a coral habitat created from living hard corals and sponges (e.g., *Porites porites*, *Meandrina meandrites*, *Desmapsamma anchorata*). In the second series of experiments the boxes were filled with rubble alone, rubble and three conspecifics, rubble and three heterospecifics, or three conspecifics alone. Rubble was used because this was the habitat where *H. flavolineatum* naturally settle at Spanish Water Bay and Piscadera Bay, and where the experimental fish were caught. Conspecifics and heterospecifics were equal in size to the experimental fish (20–30 mm) and were caught in the same rubble area. Juvenile ocean surgeonfish (*Acanthurus bahianus*) were used as heterospecifics, based on the observed presence of *A. bahianus* among schools of *H. flavolineatum* in the field.

As with the acoustical cue experiment, each fish was acclimatized within a small wire cylinder placed in the center of the cage for three minutes, and then the cylinder was removed and the fish tested for 15 minutes. Fish were monitored on release for natural swimming behavior. In no case was unnatural flight or stereotyped movement observed for test fish, or for heterospecifics/conspecifics contained in the boxes in the respective experiments. In the habitat-only experiment, 122 fish were tested, while the experiment with conspecifics was conducted with 51 fish. The time spent by each fish within 10 cm of the front glass panes of the boxes was recorded. This resulted in a total time spent at each box and remaining time in the central sandy area for each individual fish. After every two replicate experiments the cage was rotated 90° clockwise, to rule out directional environmental effects like wave motion, water currents, or the angle of sunlight. In addition, the mutual positions of the boxes were switched within the cage after four 90° turns to create a new configuration within the cage, ruling out any treatment position effects.

Behavioral arena: olfactory habitat vs. visual conspecific cues

Testing olfactory cues in situ is logistically challenging due to the high solubility of habitat-specific water in the surrounding ocean water. Therefore, we used an ex situ rectangular flow tank in which fish can switch freely between two different laminar water flows (see Appendix A), built following the design of Atema et al. (2002). A detailed description of the methods used in the flow tank experiments can be found in Huijbers et al. (2008). In this earlier study, a flow tank experiment found no differences in preferences of *H. flavolineatum* between water from the two soft-sediment vegetated habitats (mangrove and seagrass). Therefore, in the current study the response of fish to a mix of mangrove and seagrass water (bay water) compared to water collected on the coral reef was tested. Measurements of temperature found no significant difference between the two water types (independent t test, $t_{96} = 0.229$, $P = 0.819$). To test the interactive effect between visual conspecific cues and olfactory habitat cues, the flow tank was designed with transparent compartments (6×1.5 cm) on each side of the tank (see Appendix A). In each side compartment, three size-matched conspecifics (10–15 mm) were introduced after the acclimatization period of a test fish. The test fish could see but not smell conspecifics.

The fish were acclimatized for three minutes in neutral water collected from the catch location, which is located between the two habitat types (reef and bay). Subsequently, a test period of six minutes followed, during which the water type was switched between sides after three minutes (i.e., bay vs. reef and reef vs. bay). The order in which the two possible combinations of water types were offered to the fish was randomly assigned to rule out any side effects. This experiment was performed with 49 individual fish.

The swimming behavior of the fish in the different water flows was continuously recorded during the experiment. A line was drawn on the bottom of the flow tank to indicate the border between the left and right side of the tank. Dye tests showed perfect separation on this border between the two laminar water flows. Each time a fish crossed the border with >3 mm toward one side, the time and side was written down. From this, the total time spent at each side was calculated. For the final analysis, we excluded the first 30 seconds of each replicate experiment; tests with dye suggested that this was sufficient time to allow for the switch between water types in two consecutive experiments. Additionally, stressed fish that gave no response at all, and fish that stayed 100% on one side and thus might not have sensed the water type on the other side, during the first three minutes (before the water switch) of the experiment, were excluded from the analysis (12% of all fish tested).

Data analysis

Experimental data for acoustical cues were tested by use of a paired-samples t test to test the differences between time spent toward the sound (closest to the speaker) and time spent at the side farthest from the speaker. Levene's tests showed that homogeneity of variances could be assumed for all data. This analysis

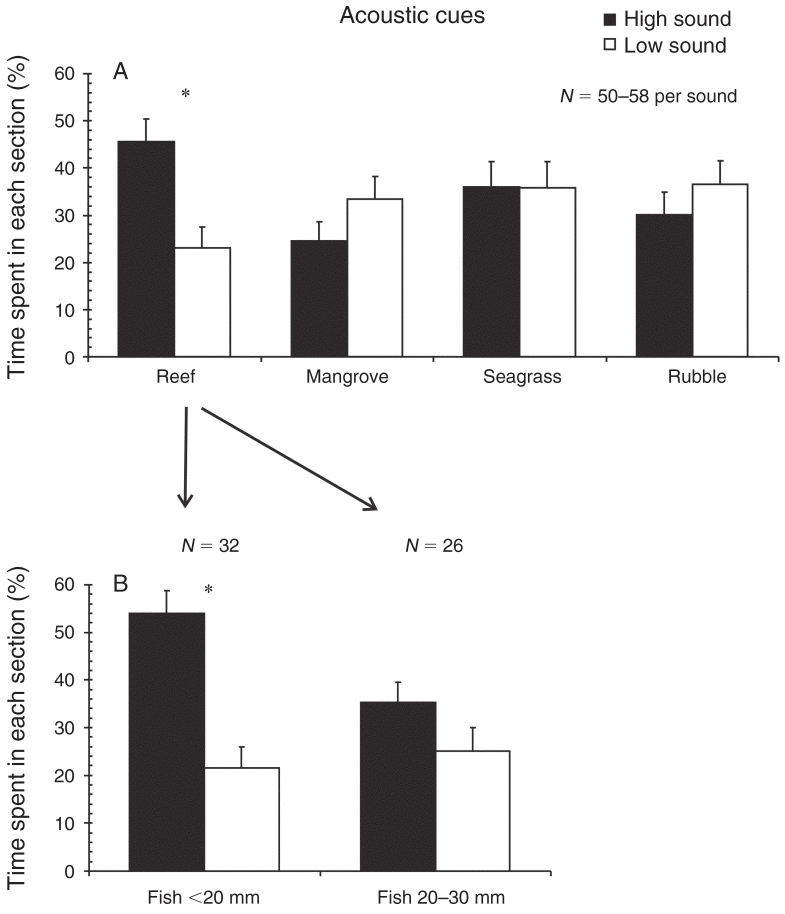

FIG. 1. (A) Mean (+SE) percentage of time fish spent in the high-sound or low-sound section of the experimental cage for each habitat-specific sound. (B) Results for coral reef sound for fish of different size classes (<20 mm and 20–30 mm standard length). * Significant differences ($P < 0.05$) among sections of the cage, tested with paired t tests.

was carried out per sound for all fish combined, and additionally with the data divided in two size classes (<20 mm and 20–30 mm) per sound.

The attraction toward visual cues was tested in a multiple choice design, and therefore repeated-measures ANOVA were performed to analyze the data. Mauchly's test confirmed that the variances of the differences between conditions were equal for the microhabitat test data, yet this assumption was violated for the conspecific cue data, and therefore a Greenhouse-Geisser correction was used to produce a valid F ratio. Bonferroni post hoc pairwise comparisons were used to further detect differences between visual microhabitat structures or conspecifics within an experiment. An independent t test was used to test for differences between the time spent in the central area between the two visual cue experiments. The habitat-only experiment was carried out with fish ranging in size between 8 and 30 mm, and these data were analyzed for any difference between small (<20 mm) and large (20–30 mm) fish. This could not be done with the data from the habitat vs. conspecifics experiment, because the size range of these fish was much smaller (17–30 mm).

To determine a response of fish to olfactory cues, a paired-samples t test was used, as the time spent in the two water flows was compared. The middle of the flow tank was not included as a choice, which is standard practice for nonresponsive animals in behavioral work (Tolimieri et al. 2004). Percentages of time spent in bay or reef water were log-transformed to meet the assumption of normality. The size range of fish tested for olfactory cues was only 10–22 mm, and therefore no distinction in size classes was used for this analysis. For all tests, the significance level was $\alpha = 0.05$.

RESULTS

Acoustic cues

Fish showed a significant attraction to coral reef noise (Fig. 1A), but other habitat-specific sounds did not induce a directional response (paired t tests between high- and low-sound sections of the cage, reef sound, $t_{57} = 2.794$, $P = 0.007$; mangrove sound, $t_{57} = -1.138$, $P = 0.260$; seagrass sound, $t_{49} = 0.003$, $P = 0.998$; rubble

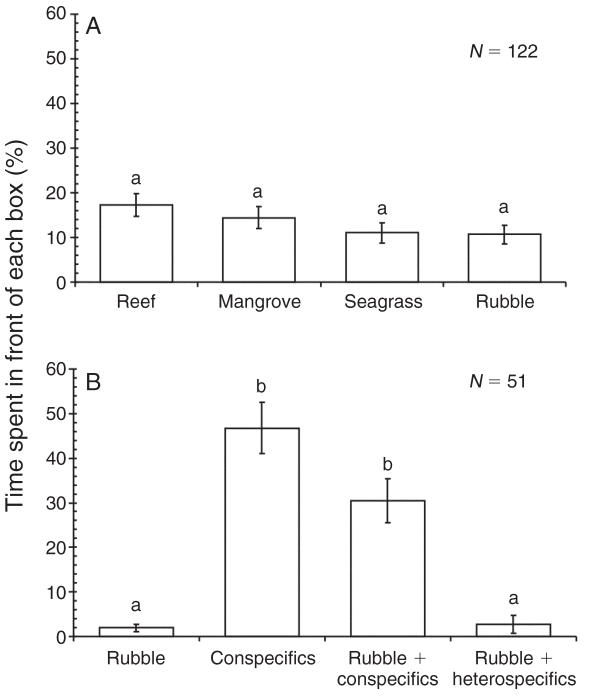

FIG. 2. (A) Mean (±SE) percentage of time fish spent in front of different microhabitat structures. (B) Mean (±SE) percentage of time fish spent in front of boxes with different combinations of microhabitat structures with or without conspecifics or heterospecifics. Different letters above bars indicate significant differences ($P < 0.05$) between boxes tested with a repeated-measures ANOVA followed by a Bonferroni post hoc test.

sound, $t_{51} = -0.745$, $P = 0.454$). For coral reef sound, a clear difference in response was observed between smaller juveniles (<20 mm), which spent significantly more time in the section nearest to the speaker during broadcasts of coral reef sounds than did larger juveniles (20–30 mm, Fig. 1B) (paired t tests between high- and low-sound sections of the cage, <20 mm fish, $t_{31} = 2.818$, $P = 0.008$; 20–30 mm fish, $t_{25} = 0.951$, $P = 0.351$). No distinctions between fish of different sizes were found for the other habitat-specific sounds (paired t tests between high- and low-sound section of the cage, mangrove sound, <20 mm fish, $t_{31} = -0.800$, $P = 0.430$; 20–30 mm fish, $t_{25} = -0.796$, $P = 0.434$; seagrass sound, <20 mm fish, $t_{24} = -0.023$, $P = 0.982$; 20–30 mm fish, $t_{24} = 0.030$, $P = 0.976$; rubble sound, <20 mm fish, $t_{25} = -1.213$, $P = 0.236$; 20–30 mm fish, $t_{25} = 0.171$, $P = 0.865$).

Visual cues

Fish did not preferentially select any of the microhabitat structures when visual cues were presented in isolation (repeated-measures ANOVA followed by a Bonferroni post hoc test, $F_{3, 363} = 1.45$, $P = 0.228$), and remained in the central sandy area of the cage for 47% of the time, on average, indicating a low attraction toward any type of microhabitat structure (Fig. 2A). This result was equal for fish <20 mm (repeated-measures ANOVA followed by a Bonferroni post hoc test, $F_{3,87} = 1.43$, $P = 0.244$) as for the larger size class, 20–30 mm (repeated-measures ANOVA followed by a Bonferroni post hoc test, $F_{3, 273} = 1.30$, $P = 0.274$). Significantly less time (18%) was spent in the central cage area when conspecifics were present than without conspecifics (independent t test, $t_{135} = 5.93$, $P < 0.001$). Visual cues from conspecifics evoked a much stronger response than the visual microhabitat cues. Fish spent more time in front of the box with conspecifics alone, although this was not significantly different from rubble with conspecifics. Rubble alone or rubble with heterospecifics did not induce a directional response (Fig. 2B, repeated-measures ANOVA followed by a Bonferroni post hoc test, $F_{3, 150} = 24.41$, $P < 0.001$).

Olfactory habitat cues vs. visual conspecific cues

In the first three minutes of the olfactory cue experiment, before water types were switched, fish showed a significant preference for bay (mangrove and seagrass combined) over reef water (Fig. 3; paired t test, $t_{48} = -2.28$, $P = 0.027$). The visual presence of conspecifics did not inhibit a behavioral response of the test fish to the olfactory cues offered.

The importance of the presence of conspecifics dominated after the sides receiving reef or bay water supplies were switched, and 55% of all fish stayed on the side of their initial choice, having associated visually with the conspecifics at that side. Test fish moved toward the middle line, and were thus able to smell both water types, but they never crossed to the water flow on the other side. Of all fish that preferred bay water in the first combination, 61% did not spend any time at all in bay water in the second combination, but remained near the conspecifics in the alternative water flow.

DISCUSSION

This study shows that early juveniles of a mangrove/seagrass-associated reef fish species are receptive to multiple sensory cues that would enable effective selection of novel habitat. Recognition of suitable habitats by post-larvae (late-stage larvae and early

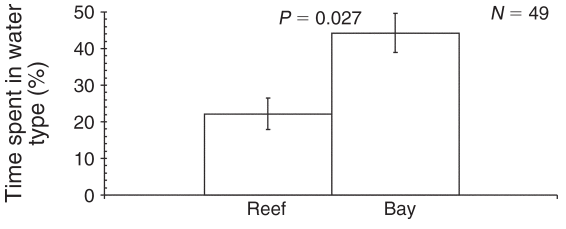

FIG. 3. Mean (±SE) percentage of time fish spent in reef or bay water with conspecifics visible at the sides, before the water was switched between sides (see *Methods: Behavioral arena: olfactory habitat vs. visual conspecific cues*). This graph represents the choice of the fish in 66% of the total time measured, as 34% of the time was spent in the middle of the tank. Levels of significance show the result of paired t tests on $\log(x + 1)$-transformed data.

juveniles) of coastal demersal species is crucial for successful settlement and recruitment (Kingsford et al. 2002). However, the mechanisms to achieve this are still poorly understood, especially for fish species that recruit primarily to nonreef habitats. The observed behavioral responses of early juveniles showed that the reaction to a particular cue was different for each sensory modality, suggesting that to avoid conflicting preferences, the selection of multiple cue types in situ could operate over different spatial and temporal scales.

Early juvenile *Haemulon flavolineatum* responded to acoustic cues from coral reefs and not from any other habitats, including their preferred initial settlement habitat (rubble, which was also the habitat in which they were collected). This is interesting, as the reef and rubble habitats shared several acoustic qualities (e.g., higher overall levels, higher low-frequency noise; see Appendix C), and suggests well-tuned acoustic discrimination capabilities. Between the two hard-substrate habitats, we intuitively expected the coral reef to have characteristically more sound than the rubble habitat. Rubble habitat may have produced more noise due to a higher density of snapping shrimp in what would be a lower-predation environment, or alternatively due to higher numbers of mobile soniferous fishes compared to the reef; however both of these hypotheses require rigorous testing in the field. The current findings suggest that the attraction to reef sound seen in these mangrove/seagrass-associated fish may be the same mechanism for orientation toward coastal environments as used by larvae of fish species returning from the pelagic environment to their settlement sites on coral reefs (Simpson et al. 2004, 2005, 2008, Leis and Lockett 2005, Montgomery et al. 2006, Radford et al. 2011).

The distance over which acoustic orientation behavior can occur will depend on the species and its stage of ontogeny, the local sources of sound and their geographic distribution (Kennedy et al. 2010), other sources of sound that may mask preferred cues, and the water conditions and bathymetry. Although there is some debate on the estimated distance at which reef cues can be detected, the general consensus appears to be that this behavior may operate over hundreds to thousands of meters (Mann et al. 2007, Wright et al. 2010). Certainly our analysis of recordings from different habitats suggests that reef noise will propagate farther than mangrove and seagrass noise, and thus may be the most valuable cue to fish returning from the open ocean if settlement-stage larvae showed a similar response toward reef noise as our early-stage fishes. In the current study, examination of the likely spatial scale of response was not tested, as the distance from the chamber to the sound source was equal for each sound, but our experimental approach could be adapted to test this in a future study. Notably, in the present study reef sound only induced a directional response for the smaller fish (<20 mm in length), which suggests that acoustical cues from the reef lose their importance some weeks to months after settlement and do not play a significant role in novel habitat detection during subsequent life stages.

In contrast to acoustical cues, differences in water chemistry elicited a response toward nonreef habitats (seagrass beds and mangroves in embayments). Fish significantly preferred bay over reef water, which is in accordance with a previous study in which mangrove and seagrass water were both preferred above coral reef water (Huijbers et al. 2008), and this initial preference was made irrespective of the visual presence of conspecifics. Several fish species are able to discriminate between different water types (Atema et al. 2002, Arvedlund and Takemura 2006, Dixson et al. 2008), and Lecchini et al. (2005) showed that organic compounds in the water can trigger such responses. Fishes did not discriminate between mangrove and seagrass water (Huijbers et al. 2008), and olfactory preference for aquatic vegetation may thus be an adaptive mechanism enabling fish to find juvenile habitats that are located past the reef.

When solely visual cues were offered, the presence of conspecifics significantly affected the behavioral choices of test fish, whereas habitat structure did not. In the first visual cue experiment testing habitat cues alone, fish spent a high percentage of the time in the central sandy area of the cage, indicating a low attraction toward a specific type of habitat structure. This concurs with other studies that have found that post-larval fish of other species do not demonstrate a preference for a specific habitat type at settlement (Tupper and Boutilier 1997, Öhman et al. 1998). However, other studies have found selectivity among habitats based on visual cues (Lecchini et al. 2005, 2007, Igulu et al. 2011), suggesting a large variety in cue use among species. During early ontogeny, fish eyes develop rapidly in the larvae of *H. flavolineatum* (McFarland and Wahl 1996), and therefore we exclude the possibility that these post-larval fish were not able to see the different microhabitat structures. In other in situ scenarios, habitat selection may be altered at small spatial scales by factors such as food availability and the presence of predators, rather than structural complexity (Adams and Ebersole 2009, Grol et al. 2011*a*). In the second visual cue experiment where conspecifics were present, the amount of time spent on open sandy habitat was significantly lower. It is a common observation in several fish species that the presence of conspecifics enhances settlement to suitable sites (Sweatman 1983, Lecchini et al. 2007). The response to conspecifics was far greater than the response to habitat structure, indicating a stronger role of conspecifics than structure during the early post-settlement phase, although a recent study showed that attraction to conspecifics decreases significantly when these occur in nonpreferred habitat (Igulu et al. 2011). To fully understand the importance of conspecific cues in addition to habitat cues, future experiments should be conducted comprising multiple habitat cues with or without conspecifics and heterospecifics.

In contrast to previous studies that have tested each cue type in isolation, we provide the first multiple-cue experiment that allowed the interactive effects of two cues to be investigated. Visual cues of conspecifics did not affect the initial preference for olfactory cues from vegetated bay habitats. Previous flow tank results (Huijbers et al. 2008) showed that switching of reef and bay (mangrove or seagrass) water to the opposite sides of the flow tank did cause the fish to move. In the current experiment, fish largely remained near to the conspecifics on the side of the initial choice based on the first water combination. Vision is considered the most dominant sense at small distances (Rowland 1999), and may thus be the best applicable sense for detailed site discrimination. The visual presence of conspecifics in our flow tank experiment may have provided both a visual and a social cue, since there could be feedback between the test fish and shoal mates, and the presence of conspecifics influenced the behavior of the test fish in such a way that the olfactory preference was subsequently overridden.

Considering multiple cue use for orientation toward novel habitats in this coral reef fish species, we propose a hypothetical scenario of stepwise cue use that could aid the return of the fish from the open ocean toward novel inshore juvenile habitats in early life. First, orientation from the pelagic zone toward the coastline is accomplished by the use of acoustical cues from noisy coral reefs. In the proximity of coral reefs, fish can locate nearby embayments that harbor mangroves and seagrass beds using olfactory cues in water plumes. Once bays are located using olfactory cues, suitable sites are then located through visual cues based on the presence of conspecifics. The mangrove/seagrass associated fish species used in this study, *H. flavolineatum*, reacted to reef sound and visual cues of conspecifics as would species that settle directly on reefs. The strong response of our test fish to olfactory soft-sediment vegetated habitat cues suggests adaptive behavior that would enable fish to locate novel inshore juvenile habitats, which has been shown to be beneficial in terms of survival rates (Grol et al. 2011*b*). Juvenile densities of this and various other mangrove/seagrass-associated species are significantly lower in areas where mangroves and seagrass beds are absent (Nagelkerken et al. 2001, Nagelkerken and van der Velde 2004, Pollux et al. 2007), highlighting the importance of preserving these habitats to facilitate orientation of fish in tropical coastal habitats.

Our finding that more than one sensory modality can be used for underwater orientation to novel habitat parallels results for aquatic species with directed navigation behavior. For example, sea turtle hatchlings orient according to both wave direction and the Earth's magnetic field (Goff et al. 1998), while in later life, juvenile turtles use visual and magnetic cues to maintain directional headings (Avens and Lohmann 2003). Salmonid fish are also able to orient using multiple cues throughout their life (Braithwaite et al. 1996, Dittman and Quinn 1996). Comparable to these studies on homing behavior, our results emphasize that multiple cues are likely integrated by early juveniles for finding novel habitat. Ontogenetic cross-ecosystem habitat shifts may increase an animal's survival, yet movement across ecosystems can generate increased risks. Directed movement and the ability to orient toward environmental cues is essential to ensure survival and arrival at an appropriate destination.

Acknowledgments

This study was financially supported by the Netherlands Organization for Scientific Research (NWO) through a VIDI grant to I. Nagelkerken. S. D. Simpson was funded by the Natural Environment Research Council, UK. We thank all staff and personnel of the Carmabi Institute on Curaçao for their hospitality and provision of research materials. We also thank J. H. F. de Brouwer, L. L. Govers, M. van de Kerk, and J. J. Oldenburger for their logistic support in the field. This manuscript was improved by comments from A. Radford, M. Meekan, and A. Heenan. This is Centre for Wetland Ecology Publication No. 542.

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Supplemental Material

Appendix A

Schematic drawing of experimental setup for testing (*Ecological Archives* E093-005-A1).

Appendix B

Details of the sound recordings that were used for playback in the acoustic cue experiments (*Ecological Archives* E093-005-A2).

Appendix C

Analysis of the sounds recorded at four different habitats (*Ecological Archives* E093-005-A3).